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ORIGINAL ARTICLE

Changes in Urinary Bladder Structure and Systemic Inflammation Response Following Incomplete Transection versus Contusion Spinal Cord Injury in Rat Model

Moosa Javdani[1*], Mohamad Hashemnia[2], Hilde de Rooster[3]

[1] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran*

[2] *Department of Pathobiology, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*

[3] *Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

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Abstract

Objective- The current study was conducted to evaluate changes in the urinary bladder structure and leukocyte profile as an important index of the systemic inflammation response for two different types of spinal cord injury (SCI) in a rat model.

Design- Experimental Study.

Animals- Forty adult healthy female Sprague-Dawley rats.

Procedure- Animals were assigned into two equal model groups: the incomplete transection group (ITG) and the contusion group (CG). In both groups, SCI was created at the T9-10 level of the column. Alterations in the urinary bladder construction and changes in the leukocytes were examined in both groups post-surgically.

Results- Degenerative changes and a reduction in the cellular volume in the mucous layer, hyperemia, and the presence of inflammatory cells in the submucosa were the most important findings in both SCI groups. The extent of the destructive lesions was more prominent in the CG 14 days after operation. At 28 days after surgery, pathological lesions including leukocyte infiltration in the submucosa, denudation of the urothelial mucosa, severe edema, atrophy of the muscle layer, and necrosis of muscle fibers in some areas were recorded in both groups; the extent and severity of the lesions were more evident in the CG. There was no significant difference between the white blood cells and N/L ratio at the different times in the CG and ITG groups.

Conclusion and clinical relevance- Despite the similar leukocyte response in the IGT and CG, more severe degenerative histological alternations in the urinary bladder structure were observed in the CG. Therefore, attention should be paid to the extent of cystitis in these patients in clinical trials and interventions.

* Correspondence to Moosa Javdani; Address: Surgery Section, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University. E-mail: m.javdani@sku.ac.ir

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1. Introduction

Traumatic spinal cord injury (SCI) induces different pathological conditions via the loss of motor and sensory nervous pathways.[1,2] The major aspects of the mentioned deficits are related to the confusion of supraspinal input, afferent input to the spinal cord, and reorganization of the intra-spinal circuitry in response to injury.[3,4] Severe deficits can be observed in the urogenital system due to SCI.[5,6] The urinary bladder can be affected structurally, physiologically, and molecularly after the incidence of SCI.[7,8]

Patients who have suffered traumatic spinal cord lesions can display sustained ulceration,[9,10] with a bladder susceptible to inflammation, chronic cystitis, and chronic bacterial infections. Hemorrhagic cystitis due to SCI has been reported in animal models.[7] It seems that SCI rapidly induces changes in the bladder uroepithelium, which include a considerable drop in the transepithelial resistance, alterations in the umbrella cell layer of the uroepithelium, and an increase in permeability to water. Most of the mentioned alternations are transient and are resolved spontaneously 28 days after SCI.[7] Inflammatory responses are a crucial element of secondary injury and participate in modulating the pathogenesis of acute and chronic SCI; they have a major role in nerve damage and play a part in the management of the regenerative reaction.[11,12]

White blood cells (WBCs) are important mediators of the inflammatory response, and, unquestionably, are the main origin of circulating pro-inflammatory cytokines.[13,14] So, evaluation of WBCs gives valuable information about the stage of post-traumatic inflammation.

Here we hypothesize that the changes in the urinary bladder structure and leukocyte profile, as an important indicator of systemic inflammation response, will be different based on the type of SCI – incomplete transection or contusion. So, the final results will help researchers to provide suitable traumatic SCI models for specific goals.

2. Materials and Methods

The present paper was designed as a supplementary scientific work of the authors based on the two researches (170-630 and 170-572) approved by the Veterinary Medicine Research Council of Shahrekord University.

Animals

A total of 40 adult female Sprague-Dawley rats weighing approximately 300g were used in the present study: 20 rats in the incomplete transection group (ITG) and 20 in the contusion group (CG). In addition, an additional group of 10 female Sprague-Dawley rats without any surgical intervention was designated as the control group and to provide basic histological and blood leukogram data.

Induction of SCI

All rats were premedicated by subcutaneous injection of xylazine (10 mg/kg; Xylazine 2%; Alfasan, The Netherlands) before any surgical and sampling interventions. Then they were induced and maintained by isoflurane inhalation anesthesia (Terrell Isoflurane; USP, USA).

In the ITG, a SCI was created at the T9-10 level of the column using an electric drill in the dorsal aspect of the spinal cord, a hemisection involved rubrospinal and corticospinal tracts bilaterally.[15] In the other group, and, at the same level, the spinal cord was compressed using aneurysm clips (Aneurysm clip, slightly curved 12 mm, MP35N; Vari-Angle McFadden) to create the contusion model.[16,17]

Postoperative consideration for the first 1–3 days included manual expression of the bladders twice daily. The animals also received enrofloxacin (10 mg/kg SC; Enrofloxacin 10%; Amineh Gostar Co., Iran) once daily for 3 days postoperatively to reduce the incidence of bladder infections.

Histopathological Assessment

At different times post-injury (day 0, 7, 14, and 28), the rats were euthanized (5 rats at each time in each group) by an overdose injection of anesthetic.[26] Following sacrifice of the animals, their urinary bladder was harvested. The bladder tissues of all the rats were embedded in paraffin following fixation in 10% buffered formalin for 24 h. Five-micrometer-thick serial sections were cut from the paraffin blocks using a microtome (Leica RM 2055; Nassloch, Germany) and stained with hematoxylin-eosin (H&E) for routine histopathological assessment at × 200 and × 400 magnifications under a light microscope (Micros Austria, MC100LED, Germany). The prepared histopathological sections were examined by an experienced histopathologist, who was blinded to the groups and study design, and were subjected to statistical analyses.

White Blood Cell Count

It has been proved that neutrophil, lymphocyte, and neutrophil to lymphocyte ratio (N/L ratio) in blood could be affected by systemic inflammatory response.[19-20] Additionally, studies have found that N/L ratio, as a reliable and cost-effective marker, has predictive value in diagnosis and treatment of some inflammatory events.[21-23] So, the blood samples were collected by heart puncture at days 7, 14, and 28 immediately before sacrifice of the animals, and the total and differential WBC count were measured manually from thin Wright-stained blood smears (expressed as 10^6 /L), and N/L ratio were calculated.[24,25]

Statistical Analysis

Due to the normal distribution of data, the means of the measured parameters (mean ± SEM) were compared between the ITG and CG groups using an unpaired t-test. Data alternations in each group were analyzed using the Kruskal-Wallis test. $p < 0.05$ was considered statistically significant.

3. Results

Histopathological Findings

On microscopic examination, the normal bladder showed tightly packed muscle bundles separated by narrow bands of connective tissue. Many muscles were cut in an oblique rather than longitudinal or transverse orientation as a result of the complex interwoven nature of the muscle bundles in the bladder. The urothelium normally consisted of several cell layers and possessed a regular, polarized architecture of increasing morphological complexity and differentiation from base to surface (Figures 1A-C). The urinary bladder of the experimental animals in both groups that received SCI showed various pathological lesions when compared with the control. At 7 days post operation (DPO), surgical intervention resulted in some alterations in all layers of the urinary bladder in both groups. Degenerative changes and reduction of cellular volume in the mucous layer, hyperemia, edema and the presence of inflammatory cells in the submucosa, and an increase in collagen between the atrophic muscle bundles were the most important findings in both SCI groups.

Fourteen days post operation, a decrease in the size of some, but not all muscle cells was observed in both the experimental groups. Changes in the morphology of the urothelium including the disappearance of apical cells in some areas, necrosis, and disorganization of the cell layers were prominent. Moreover, the submucosa was edematous, and numerous dilated blood vessels in the superficial lamina propria, as well as infiltration of inflammatory cells including neutrophils and less lymphocytes and macrophages, were observed. The extent and severity of the lesions were more prominent in the contusion group. At 28 DPO, the pathological lesions in the incomplete transection group included remarkable thickening of the submucosa due to severe edema and hyperemia and congestion of the vessels, infiltration of neutrophils and lymphocytes in the submucosa but less in the muscularis

layer, and necrosis of individual muscle cells. At the same time, complete denudation of the urothelial mucosa, severe hyperemia and edema and extensive hemorrhage in the mucosa and submucosa layers, severe atrophy of the muscle layer, massive necrosis of the muscle fibers in some areas, severe infiltration of neutrophils cells in the submucosa and muscularis layers, and also accumulation of pus in the bladder were evident. Specifically, the extent and severity of lesions in the contusion group were greater than that for the incomplete transection group (Figure 1).

Leukogram Analyses

The means (± SEM) of the total white blood cell (tWBC) and neutrophil-to-lymphocyte (N/L) ratio for the different times in the CG and ITG groups are shown in Table 1. There is no significant difference between these parameters for days 7, 14, and 28 of the investigation ($p > 0.05$).

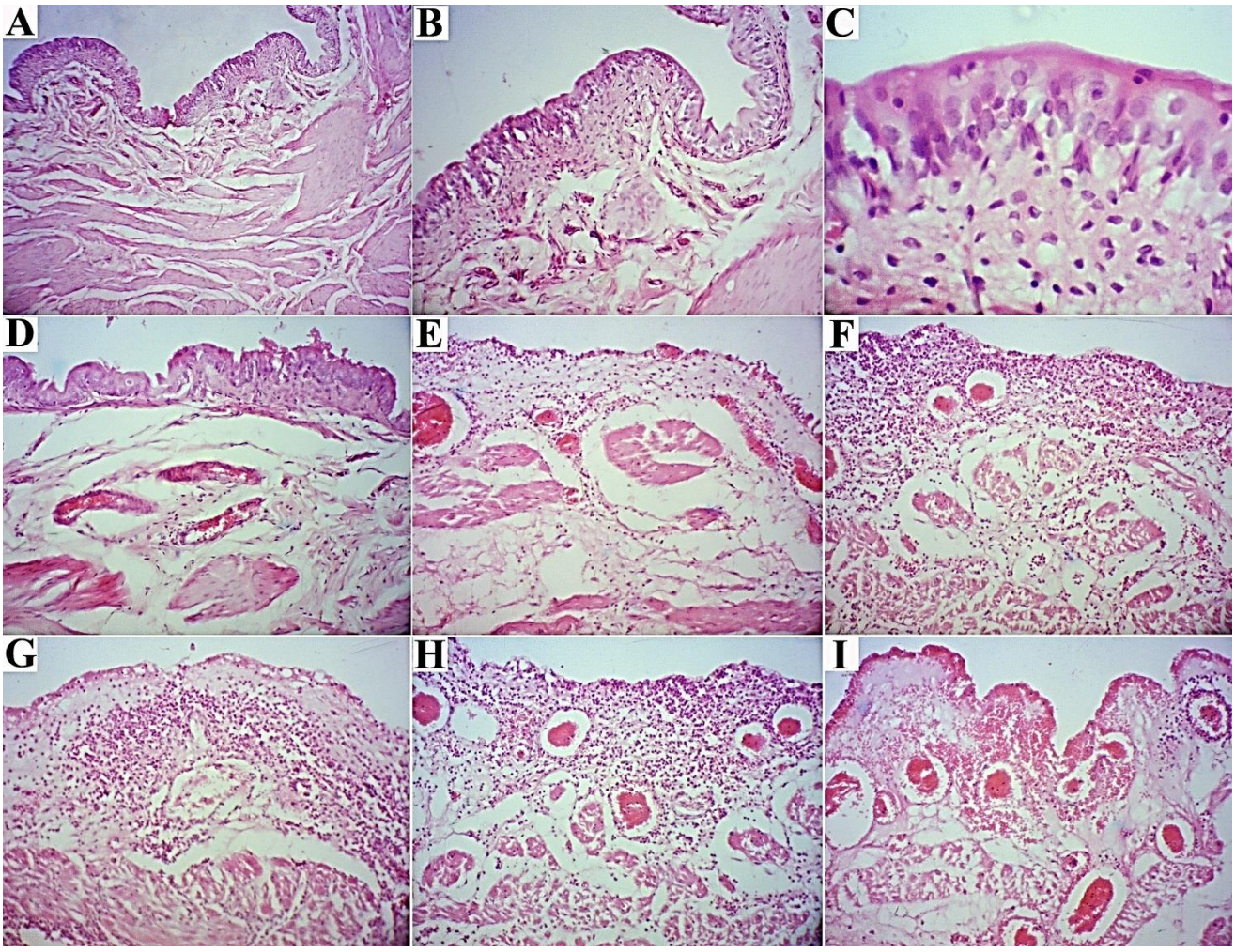

Figure 1. Urinary bladder sections of the control and experimental groups. A-C) Normal architecture of urinary bladder (H&E Staining; Bars = 150 µm (A), 50 µm (B), 20 µm (C)); D) Reduction of cellular volume in the mucous layer, hyperemia and submucosa edema (H & E; Bar = 50 µm); E) Necrosis and disorganization of cells in mucosal layer, numerous dilated blood vessels in the lamina propria and moderate infiltration of inflammatory cells in the submucosa layer (H & E; Bar = 50 µm); F) Denudation of the urothelial mucosa, edema, hyperemia, severe neutrophils infiltration in the submucosa layer and atrophied muscle fibers (H&E; Bar = 50 µm); G) Urothelial necrosis and the presence of inflammatory cells in the submucosa (H&E; Bar = 50 µm); H) Denudation of the urothelium, severe infiltration of inflammatory cells in the submucosa and muscularis layers (H & E; Bar = 50 µm); I) complete denudation of the urothelial mucosa, extensive hemorrhage in the mucosa and submucosa and massive necrosis of muscle fibers (H & E; Bar = 50 µm).

Table 1. Comparison of white blood cell count (10^6/L) and N/L ratio in the CG and ITG groups.

Day		CG	ITG	*p*-value
0	tWBC	6890±700.5	7000 ± 912.87	0.68
	Lymphocyte	4938±415	5256±505.6	0.4
	Monocyte	350±134.2	370±140	0.29
	Neutrophil	1370±988	1408±1000.5	0.66
	N/L ratio	0.30 ± 0.04	0.28 ± 0.11	0.54
7	tWBC	9920±881.5	8250±853.91	0.30
	Lymphocyte	6424±1407	5960±887.4	0.61
	Monocyte	1730±1381	385±160.7	0.05
	Neutrophil	2105±1391	1855±988.2	0.06
	N/L ratio	0.35±0.27	0.34±0.08	0.71
14	tWBC	10285±830.25	10250±1250	0.11
	Lymphocyte	5200±999	5975±445.5	0.41
	Monocyte	900±201.8	600±198	0.14
	Neutrophil	4170±1150	3610±1050	0.06
	N/L ratio	0.80±65	0.62±0.12	0.07
28	tWBC	9520±675.8	10000 ± 158.11	0.4
	Lymphocyte	6315±532.9	6915±564	0.11
	Monocyte	745.5±369.2	770±114.6	0.24
	Neutrophil	2290±396	2260±897.5	0.39
	N/L ratio	0.37±0.9	0.35 ± 0.03	0.55

Values are expressed as means ± SEM; p-value < 0.05 indicates significant difference in each row; CG: Contusion group, ITG: Incomplete transection group, tWBC: Total white blood cell, N/L ratio: Neutrophil/Lymphocyte ratio

4. Discussion

The current study was conducted to compare the histological changes in the bladder structure for four weeks due to two different types of SCI – IGT and CG – and also changes to the white blood cells following these injuries. Similar to previous studies,[7,8] the present study confirmed bladder epithelium disruption as a secondary sequelae resulting from the SCI. In fact, both forms of SCI, incomplete transection and contusion, can introduce unfavorable histological changes in the rats suffering from SCI. The current histological findings showed inflammatory and degenerative alternations in both groups 7 days after the SCI while 2 and 4 weeks after the SCI the extent and severity of the observed lesions were more prominent in the contusion group. Notwithstanding the study of Apodaca *et al*., our results showed that these structural alternations, especially in the CG, are not transient and do not resolve during the 28 days after spinal damage. Evaluation of the histological sections and the presence of pus 28 DPO, clearly revealed bacterial cystitis, which, previously, is mentioned as a notable complication in patients with SCI.[27] Early disruption of the uroepithelial integrity due to clinical traumatic spinal cord damage creates hematuria, a loss of transepithelial resistance of the bladder, and increases its permeability to both water and urea.[7] It seems that the breakdown of the bladder epithelium and weakness of the deep layers results in an invasion of bacteria into the deep structures, and, finally, chronic bacterial cystitis.

Any trauma to the spinal cord induces a noticeable release of neurotransmitters, such as catecholamines, which may affect the uroepithelial integrity,[28] as the subsequent presence of urine in the deep layers could stimulate a cellular inflammatory response and intensify the deterioration process of the bladder structure.[7] An acute SCI can disrupt bladder innervation and cause bladder atony, urine retention, and urine incontinence. Such a neurogenic bladder will be a suitable environment for the development of urinary infection.[29] The clinical evaluation of the current study, concurrent with the histopathological examination, confirmed hematuria but these macro- and microscopic alternations in the ITG were chiefly restored to near normal levels by 28 days post-trauma. This recovery process was not observed in the CG. Functional recovery after incomplete injuries results from the plastic nature of the brain and also spinal cord. In fact, local structural rearrangements of the spinal cord as a compensatory mechanism regenerate partial SCI.[30,31] This neuronal recovery may explain the relative rehabilitation of bladder damage in the ITG of the present study. As the other investigations indicated, SCI results in an increase in the inflammatory cells in the systemic circulation and activates the immune cells, which affect different organs after SCI.[32-34] Likewise, our blood works demonstrated a significant increase in the total white blood cells after SCI, especially at 14 and 28 DPO. Based on the presented data, no significant differences were observed between the total

and the differential WBC count, or the N/L ratio in the ITG and CG; any type of trauma to the spinal cord results in an infusion of WBCs to the bloodstream. As well documented, the N/L ratio is a fast, cheap index of systemic inflammation and stress.[35] In the current paper, no notable changes were observed between the postoperative measured N/L ratio and its basic level. Nevertheless, our previous study showed a significant increase in the N/L ratio 3 DPO. In both groups, neutrophils increased days after SCI, especially day 14 DPO.[26] These blood cells are crucial elements for functional well-being after spinal cord damage.[36] In conclusion, despite the similar leukogram response as a main systemic inflammatory response in the IGT and CG, more severe negative histological alternations and degenerative processes in the urinary bladder structure were observed in the CG. Therefore, attention to the severity and extent of induced cystitis in any contusion model of SCI during any related researches and therapeutic intervention is necessary.

Conflict of Interests

None.

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